West Virginia Univ.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

PROGRESS REPORT

Report Prepared by:	Walter H. Moran, Jr., M.D.
For Period:	August 14, 1967 to February 15, 1968
Grant:	NGR-49-001-019
Title:	The Effect of Changing Gravity and Weightlessness on Vasopressin Control Systems

A. FACILITIES AND PERSONNEL:

Drs. Choon Kim and Derek Hunter have joined the research unit as post-doctoral fellows and are involved in the biological half life and tilt experiments, respectively. Mr. Jay Hopper, a graduate fellow working on his masters degree in electrical engineering, is continuing our work with digital logic for the LINC-8. All other personnel remain the same.

B. LINC-8 OPERATING SYSTEM:

The following programs have been written to improve the usefulness of the LINC-8 in our research program. These programs are of general interest to anyone using the LINC-8 computer.

1. DATUM-8

DATUM-8 is a general purpose, data manipulation program which allows one to fetch and display graphic information stored in a block of LINCtape. This data can then be measured by cursor pens or manipulated by addition, subtraction, filtering, differentiation, integration and the graphic result stored back on the LINCtape. A special feature of this program is its ability to integrate between two adjustable cursors

2. LEAP LINCtape Operating System

H.L. Pearson has modified the PDP-8 DECtape operating system written by John Fitzgerald to allow its use of the LINCtapes of the LINC-8. This system provides an efficient method of assembling PDP-8 programs through the use of the high speed magnetic tapes, instead of paper tape. The use of LEAP and the recently released LAP-6 written by the St. Louis group, greatly reduces the time spent in program development.

C. ANTIDIURETIC HORMONE (ADH) ASSAY:

The following improvements have been made in the ADH assay procedure.

1. Isolation of ADH with Paper Chromatography

The technique of paper chromatography used in the isolation of ADH was described in the last report. This chromatographic system isolates ADH from oxytocin, bradykinin and angiotensin. It does not separate arginine vasopressin (AVP) from lysine vasopressin (LVP), through mixtures of these two substances produce a bilobed peak. The sharpness of the arginine vasopressin peak is illustrated in figure 1.

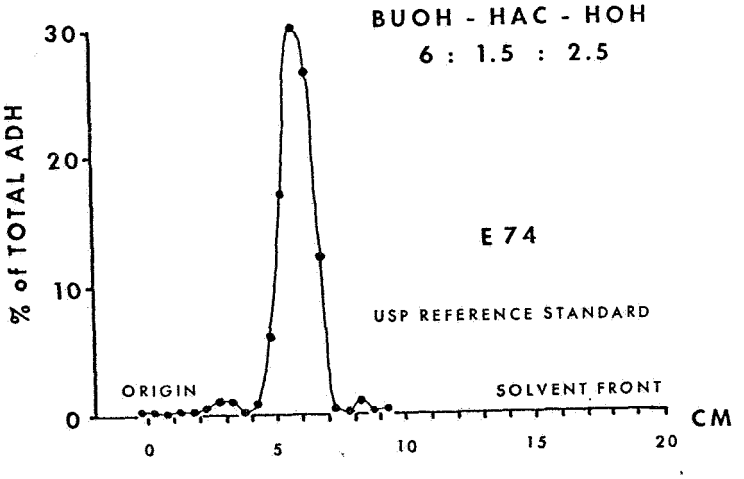

Fig. 1 Distribution of 12 ng of AVP after a 3½ hour paper chromatographic run.

This system has not become a routine part of the method, as yet, but is being used in the study of the diuretic peaks.

2. On-line Analysis of Rat Bioassay Signals with LINC-8

A program called SAMRAT has been developed to monitor changes in urinary conductance (G) and urinary rate (V) produced during the ADH bioassay procedure. This program also computes and displays the voltage analogs of osmolal clearance (C_{osm}) and free water clearance (C_{HOH}) as they develop in time. One may extract the actual value of any of these parameters by use of a pen cursor. The graphic information can be stored on LINCtape for further manipulation and analysis. The typical response of G, V, C_{HOH}, and C_{osm} to ADH standards is illustrated in figure 2.

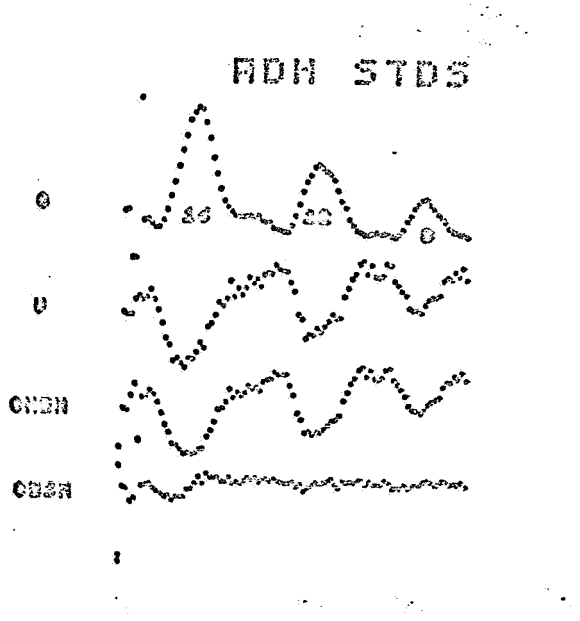

Fig. 2 Response to 16, 12, and 8 μU doses of ADH standard.

Note the slight depression of C_{osm} with subsequent rebound that occurred with the 16 μU standard. The response of these parameters to ADH standards was analyzed (Fig. 3, 4). It appears that the log response vs. dose relationships are the most satisfactory.

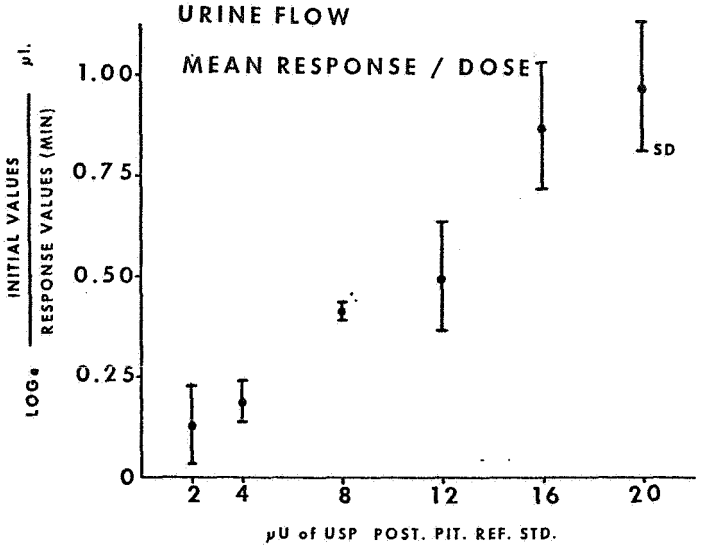

Figure 3

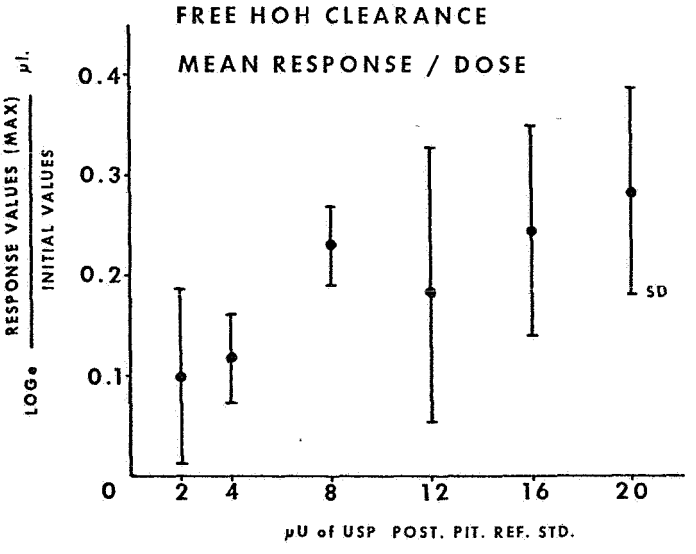

Figure 4

The C_{osm} usually did not change making the log G response/dose relationship a valid parameter also.

D. DIURETIC HORMONE:

The immediate and transient diuresis observed during assay to the plasma samples from Vietnam has been seen again in dogs under going acute tilting experiments. This response, which we at first considered an aritfact, may have some biologic significance. The substance responsible may be the diuretic hormone described by Gauer or the natriuretic substance described by Cort.

We have decided to apply the LINC-8 and its program SAMRAT to the systematic study of these transient diuretic responses. Figure 5 illustrates the sharp increase in V following the injection of 800 µl of plasma extract from sample 2715. Note the concomitant transient increase in C_{HOH} and prolonged increase

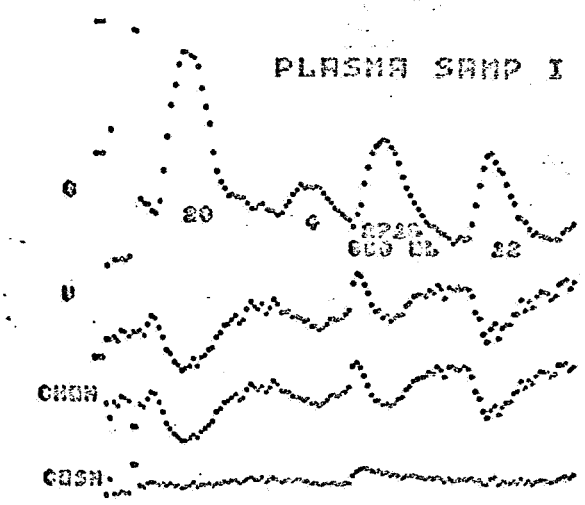

Figure 5

in C_{osm}. These changes did not occur with ADH (Figs. 2, 5, and 6). These responses occurred with smaller volumes from plasma sample 2723 (Fig. 6). These changes occurred in all plasma samples of this tilt experiment.

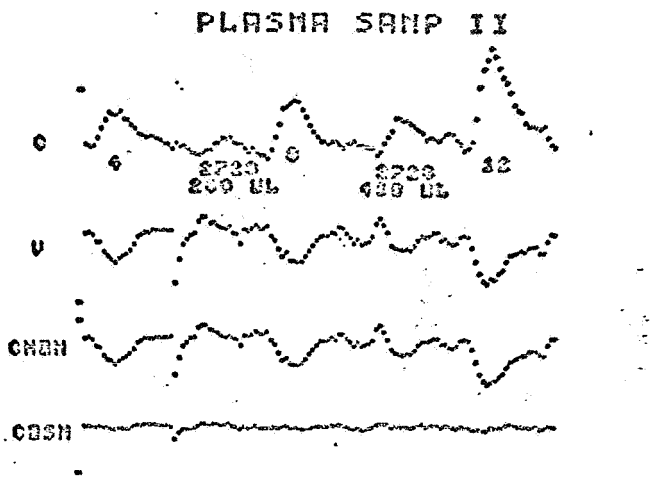

Figure 6

In order to be sure that this substance is a separate entity, we will attempt to separate this response from that of ADH by ion exchange and paper chromatography.

E. PUBLICATIONS:

1. Young, R.W.: DECUS No. L-11, DATUM8, Decuscope 6(6): 43-C, 1967. (abst)

2. Ukai, M., Moran, W.H., Jr., Zimmermann, B: The role of visceral afferent pathways on vasopressin secretion and urinary excretory patterns during surgical stress, Ann. Surg. (In Press)

APPENDIX I

LIST OF EXPERIMENTS SUPPORTED BY NGR-001-019
THROUGH 2/14/68

E 19	ADH STABILITY I, EFFECT OF TEMPERATURE
E 20	EFFECT OF BLEED ON ADH AND CRF
E 21	ADH STABILITY II, EFFECT OF REPEATED FREEZE-THAW
E 21	ADH STABILITY II, REPEATED FREEZE-THAW PLASMA
E 22	ADH STABILITY III, REPEATED FREEZE -THAW STANDARD SOLUTION
E 23	ADH STABILITY IV, EFFECT OF TEMPERATURE
E 31	ANTIDIURETIC ACTIVITY OF ANGIOTENSIN
E 32	ANGIOTENSIN RECOVERY ON CG-50 RESIN COLUMNS
E 33	ADH STABILITY V, NA OXALATE AND EDTA AS INHIBITORS
E 34	ADD RECOVERY I, NO ETHER WASH
E 39	ADH STABILITY VI, NA OXALATE AND EDTA AS INHIBITORS
E 40	CRF ISOLATION I, TO AMES
E 41	CRF ISOLATION II, TO AMES
E 42	ADH RECOVERY II, NO ETHER WASH
E 43	ADH RECOVERY III, PH 4.0 RESIN COLUMN
E 44	ADH RECOVERY IV, EE VS NEE
E 45	ADH RECOVERY V, EE VS NEE
E 46	ADH RECOVERY VI, EE VS NEE
E 47	CRF ISOLATION III, TO AMES
E 49	VEITNAM SAMPLES, CRF TO AMES
E 50	ADH ISOLATION I, DIURETIC PEAK
E 53	ADH ISOLATION II, NAOX AND EDTA ON DIURETIC PEAK
E 54	ADH RECOVERY VII, PLASMA
E 55	ADH ISOLATION III, NA ION IN EXTRACT
E 57	ADH ISOLATION IV, THIOGLYCOLATE CORD BLOOD
E 58	ADH ISOLATION V, THIOGLYCOLATE CORD BLOOD
E 61	ADH ISLOATION VI, PITRESSIN VS. USP POST PIT REF IN RAT
E 63	ADH STABILITY VII, STORAGE OF DILUTED EXTRACT INJECT SOL
E 64	ADH CHROMATOGRAPHY I, PITRESSIN
E 65	ADH CHROMATOGRAPHY II, ELUTION FROM PAPER
E 66	ADH CHROMATOGRAPHY III, USP PIT REF
E 67	ADH CHROMATOGRAPHY IV, RESIN + PAPER WITH USP REF
E 68	ANGIOTENSIN CHROMATOGRAPHY I,
E 69	OXYTOSIN CHROMATOGRAPHY I,
E 71	CRF ISOLATION IV, TO AMES
E 72	ADH CHROMATOGRAPHY V, CORD BLOOD
E 73	ADH RECOVERY VIII, RESIN PLUS PAPER CHROMATOGRAM
E 74	ADH CHROMATOGRAPHY VI, USP REF STD - DIST COEF
E 75	ADH CHROMATOGRAPHY VII, CG-50 ION EXCHANGE COL
D 7000	BIOLOGICAL HALF-LIFE OF ADH, DOG
D 7001	BIOLOGICAL HALF-LIFE OF ADH, DOG
D 8000	EFFECT OF TILT ON ADH SECRETION
E 76	ADH RESPONSE I, URATE, CHOH, COSM VS. DOSE